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(74) Agent: HAILE, Lisa, A.; Gray Cary Ware & Freidenrich
LLP, Suite 1600, 4365 Executive Drive, San Diego, CA
92121 (US).

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(71) Applicant (*for all designated States except US*): AURORA
BIOSCIENCES CORPORATION [US/US]; 11010 Tor-
reyana Road, San Diego, CA 92121 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): STACK, Jeffrey,
H. [US/US]; 6619 Edmonton Avenue, San Diego, CA
92122 (US). WHITNEY, Michael [US/US]; 5171 Frink
Avenue, San Diego, CA 92117 (US). CUBITT, Andrew,
B. [US/US]; 11083 Caminito Encanto, San Diego, CA
92131 (US). POLLOK, Brian, A. [US/US]; 18283 Sun
Maiden Court, San Diego, CA 92127 (US).

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ning of each regular issue of the PCT Gazette.*

(54) Title: METHODS OF PROTEIN DESTABILIZATION AND USES THEREOF

(57) Abstract: This invention is directed towards methods of destabilizing proteins in living cells, and their use for the development of novel assays. In one embodiment, the invention comprises the use of non-cleavable multimerized ubiquitin fusion proteins to destabilize a target protein, such as a reporter moiety. In one aspect of this method the constructs also comprises a linker that operatively couples the reporter moiety to the multimerized ubiquitin fusion protein. In this embodiment, enzymatic modification of the linker results in a modulation of the coupling of the reporter protein to the multimerized ubiquitin domains resulting in a change in the stability of the reporter moiety. The level of the reporter moiety in the cell can then be used as a measure of the enzymatic activity in the cell. In another embodiment the invention provides for a generalized way of coordinately regulating the cellular concentration of a plurality of target proteins. In one aspect of this method, the target proteins are operatively coupled to a ubiquitin fusion protein via linker containing a protease cleavage site. Cleavage of the linker by a protease results in uncoupling of the target protein from the multimerized ubiquitin construct, and results in an increase in the stability and concentration of the target protein.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/03791

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12Q1/37 C12Q1/34 G01N33/532 C12N15/00 G01N33/50
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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12Q G01N C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 54348 A (CLONTECH LAB INC) 28 October 1999 (1999-10-28) page 7, line 1 -page 9, line 10 ---	1-76
X	DEICHSEL HEIKE ET AL: "Green fluorescent proteins with short half-lives as reporters in Dictyostelium discoideum." DEVELOPMENT GENES AND EVOLUTION, vol. 209, no. 1, January 1999 (1999-01), pages 63-68, XP002191581 ISSN: 0949-944X the whole document ---	1-76
X	US 5 503 977 A (JOHNSSON NILS ET AL) 2 April 1996 (1996-04-02) abstract; figure 1 --- -/--	1,9



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

* & * document member of the same patent family

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Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hart-Davis, J

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 01627 A (IGEN INC ;YOST PAMELA B (US)) 16 January 1997 (1997-01-16) the whole document	23-32, 34-40, 42-53, 55-58, 60-62, 64-66, 68-70
X	WO 88 02406 A (MASSACHUSETTS INST TECHNOLOGY) 7 April 1988 (1988-04-07) the whole document	23-32, 34-40, 42-53, 55-58, 60-62, 64-66, 68-70
P,X	STACK JEFFREY H ET AL: "A ubiquitin-based tagging system for controlled modulation of protein stability." NATURE BIOTECHNOLOGY, vol. 18, no. 12, December 2000 (2000-12), pages 1298-1302, XP002191582 ISSN: 1087-0156 the whole document	1-76
P,X	DANTUMA N P ET AL: "SHORT-LIVED GREEN FLUORESCENT PROTEINS FOR QUANTIFYING UBIQUITIN/PROTEASOME-DEPENDENT PROTEOLYSIS IN LIVING CELLS" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 18, May 2000 (2000-05), pages 538-543, XP002945558 ISSN: 1087-0156 the whole document	1-76

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 77-79

Present claim 77 relates to test chemicals defined solely by reference to a desirable characteristic, namely that they may be identified by the methods of claims 72-76. Claims 78-79 relate to pharmaceutical compositions comprising such identified test chemicals.

No technical features of the substances are present in claims 78-79 which would lead to desirable (pharmaceutical) properties, the technical features formulated so as to permit the execution of a meaningful search. The very broad range of compound types given as possible "test chemicals" on page 15, line 33 to page 16, line 4 does not provide a disclosure (Article 5 PCT) enabling the skilled man to carry out the invention to which claims 77-79 relate. Claims 77-79 could be considered to be partially supported (Art. 6 PCT) and partially disclosed (Art. 5 PCT) in as much as the description describes tests with capase inducers and inhibitors (page 67, lines 12-21) and with radicicol and geldanamycin, inhibitors of HRV 2A protease activity (page 71, lines 2-20). However, in both cases, the compounds in question are known. In effect, claims 77-79 provide no means for distinguishing known substances from novel substances. No definition of the subject matter for which protection is sought is therefore derivable from these claims (Article 6 PCT) or the description (Article 5 PCT).

Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

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PCT/US 01/03791

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9954348	A	28-10-1999	US 6130313 A AU 1409099 A EP 1071706 A1 WO 9954348 A1 US 6306600 B1	10-10-2000 08-11-1999 31-01-2001 28-10-1999 23-10-2001
US 5503977	A	02-04-1996	AU 2288595 A WO 9529195 A1 US 5585245 A	16-11-1995 02-11-1995 17-12-1996
WO 9701627	A	16-01-1997	AU 6290496 A WO 9701627 A1	30-01-1997 16-01-1997
WO 8802406	A	07-04-1988	CA 1313831 A1 EP 0324789 A1 JP 2776414 B2 JP 8252096 A JP 2883594 B2 JP 10057061 A JP 3038193 B2 JP 11187875 A JP 2501618 T JP 2612874 B2 WO 8802406 A2 US 5132213 A US 5496721 A US 5093242 A US 5646017 A US 5847097 A US 5196321 A	23-02-1993 26-07-1989 16-07-1998 01-10-1996 19-04-1999 03-03-1998 08-05-2000 13-07-1999 07-06-1990 21-05-1997 07-04-1988 21-07-1992 05-03-1996 03-03-1992 08-07-1997 08-12-1998 23-03-1993